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Progress in the Synthesis of OPC-15161: Easy Access to Dioxygenated Pyrazine *N*-Oxide Structure

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Abstract:

An improved synthetic route to OPC-15161 (1), a novel inhibitor of superoxide anion generation, is described. Choice of the protecting group is the key to the second-generation synthesis. Usefulness of the 2-cyanoethyl (CE)-protecting group in our process research is emphasized in comparison with that of other protecting groups. This process can be carried out in four steps with 40% overall yield from tryptophan methyl ester, which also opens a general route for the preparation of the related 5-alkoxypyrazin-2(1*H*)-one 4-oxides.

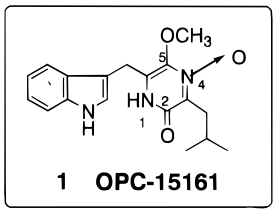
Introduction

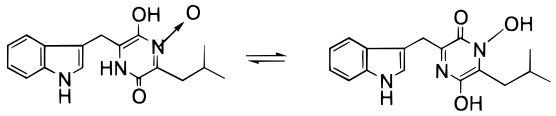
Superoxide anion released by macrophages or neutrophils may produce tissue damage involved in ischemic or inflammatory processes, and hence, inhibitors of superoxide anion generation may be effective in protecting against tissue damage in in vitro and in vivo models of ischemia and inflammation. OPC-15161 (**1**) has emerged as a novel inhibitor against superoxide anion generation, isolated in our institute as a major degradation product of the fungal metabolite OPC-15160 (a dimeric compound of **1**).[1] The compound **1** therefore has promising therapeutic potential upon the above-mentioned disorders. There is urgent necessity to find an efficient and economical synthetic route to **1**, so that we can further evaluate and develop it as a useful therapeutic agent.

Since an oxidation-labile indole ring and a highly oxygenated pyrazine ring are incorporated in the same molecule, it was difficult to achieve selective *N*-oxidation of pyrazine ring without damaging the indole ring. (Some unsuccessful transformations are summarized in the Scheme 1.)

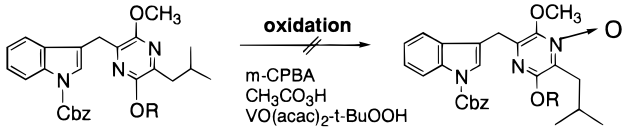
Under these circumstances, three chemical syntheses of **1** have been attained independently by Ito, Kita, and Ohta.[2] As summarized in Scheme 2, Ito announced the first total synthesis (13 steps). Later, Kita opened the shortest pathway (four steps), although there were drawbacks to be considered: (1) low regioselectivity in the methylation (22%) and (2) necessity of an expensive Meerwein reagent. These are both involved in the methylation reaction of the pyrazine tautomers (Scheme 1).[2c,3]

Scheme 1



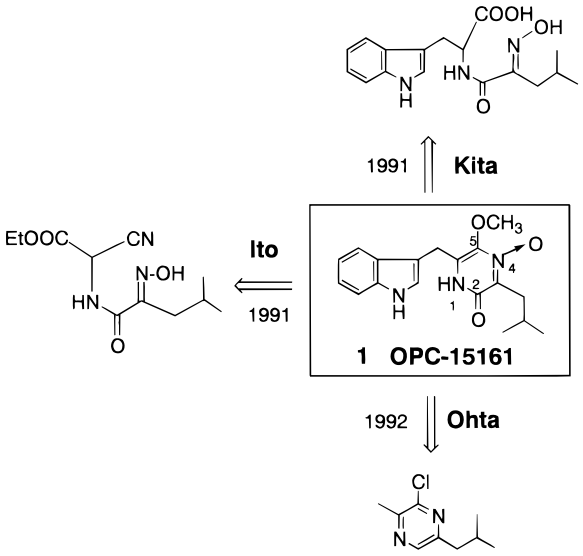


Attempted *N*-Oxidation



R= H, Ac, Bn, TBDMS, TBDPS

Scheme 2. **Previous synthetic routes**



We would like to disclose herein an improved synthetic route to **1** with detailed experimental procedures, which overcomes the obvious disadvantages mentioned above and

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(1) Nakano, Y.; Kawaguchi, T.; Sumitomo, J.; Takizawa, T.; Uetsuki, S.; Sugawara, M.; Kido, M. *J. Antibiot.* **1991**, *44*, 52−58.

(2) a) Ito, Y.; Sato, H.; Murakami, M. *J. Org. Chem.* **1991**, *56*, 4864−4867. (b) Kita, Y.; Arai S.; Fujioka, H.; Tamura, Y.; Tone, H.; Taniguchi, Y. *Tetrahedron Lett.* **1991**, *32*, 6019−6020. (c) Kita, Y.; Arai S.; Fujioka, H.; Tamura, Y.; Tone, H.; Taniguchi, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 875−884. (d) Jing, H.; Murakami, K.; Aoyagi, Y.; Ohta, A. *Heterocycles* **1992**, *34*, 1847−1856.

(3) Conventional methylation of the tautomers led to a mixture of the two *O*-methylated products.

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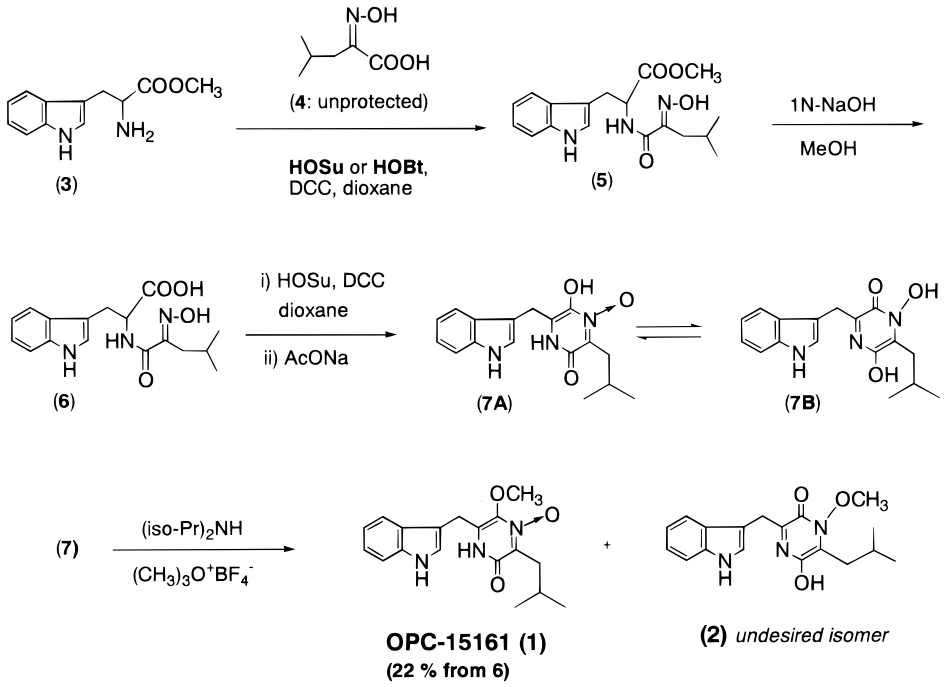
Scheme 3. **Route 1: initial plant-scale synthesis**



thus opens a route for the industrial production of such important pyrazines.

Results and Discussion

Initial Results. Our initial attempt at plant-scale synthesis was carried out by the proper modifications of Kita's route (Scheme 3). A whole sequence of reactions was carried out without protecting groups. In brief, the crucial cyclization was carried out to convert the oxime acid **6** into **7** [active ester formation by *N*-hydroxysuccimide (**HOSu**) followed by cyclization with DCC].[4] Methylation of **7** with Meerwein reagent then furnished the title compound **1** in 22−29% yield. However, the undesired isomer (**2**) was always produced as a major product (**2**/**1** = 2/1). Among the various methylation reagents examined, an aryl methyltriazene (such as 3-methyl-1-*p*-tolyltriazene)[5] showed unique regioselectivity; it afforded **1** as a major product in 39% yield. The obvious drawback with the aryl triazenes is their explosive character and mutagenic nature. Therefore, initial plant synthesis employed expensive Meerwein reagent in the key methylation reaction. Fortunately, **1** was easily isolated as a DMF adduct from the reaction mixture without using chromatography, albeit at low yield. This first plant synthesis eventually required a lot of batches due to the difficulty of controlling large-scale reaction.

Towards Second-Stage Process: Strategy. We next focused on the protection of the N^1-amide nitrogen in order to attain the selective methylation reaction in our second-stage plant synthesis. In general, protection-deprotection sequence requires extra steps and may give side-reactions. Thus, it was not so easy for us to judge whether *N*-protection was really fruitful until we found the ultimate protecting group. For the protection of N^1-nitrogen, we started with a conventional *N*-benzyl group. This, however, led to an incomplete reaction in deprotection, even under forced hydrogenolysis conditions. Our next attempts were focused on the utility of the 2,4-dimethoxyphenylmethyl (**DMPM**) group, which is easily removable under normal acidic conditions.

For the oxime protection, an easily removable protecting group was required. We found that the attachment of the 2-cyanoethyl (**CE**) group on the oxime was exceptionally beneficial. Compared with benzyl- and allyl-protecting groups, deprotection of CE and hydrolysis of methyl ester were possible at the same time, under usual alkaline conditions from **5** to **6** as described in the Scheme 4. This CE-protection was essential for the preparation of **5** via the acid chloride intermediate. Other candidates were also considered and investigated briefly, but important progress was obtained in the following three cases.

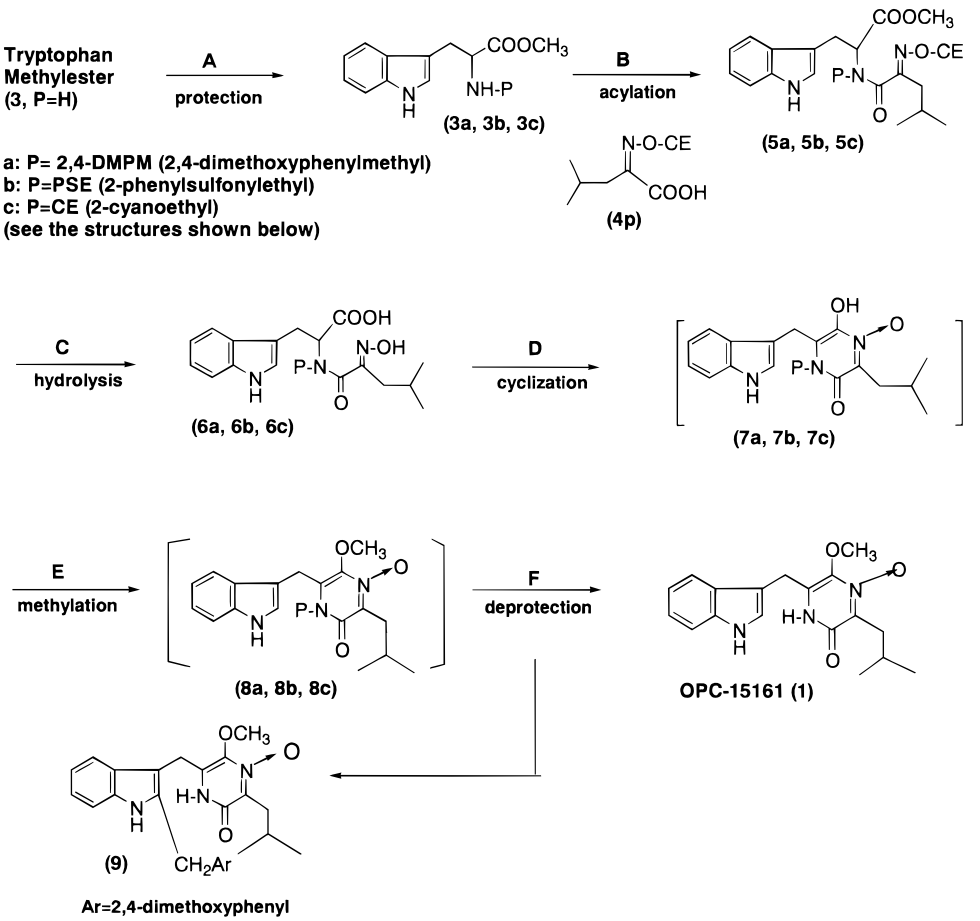
Protection by 2,4-Dimethoxyphenylmethyl (DMPM) Group. By the attachment of the protecting group, two beneficial effects soon emerged. First, the cyclization reaction of **6a** under the usual conditions (DCC−HOSu in DMF) proceeded smoothly without any additional bases (e.g., AcONa)[4] to afford the expected product **8a** in 36% yield after treatment with CH_2N_2.Thus, increased nucleophilicity of the oxime nitrogen for cyclization was realized by the attachment of the protecting group. Second, as a more convenient choice for the methylation step, it was found that addition of K_2CO_3 and $(CH_3)_2SO_4$ instead of CH_2N_2 was successful to give **8a** in 64% yield. For the smooth

(4) In the case of the unprotected substrate (**6**), reaction with DCC−HOSu stopped at the stage of succinimidyl ester, and thus, base (AcONa or KF) was added for further cyclization. Other condensing agents (e.g., NEt_3−ClCOOEt, CDI) were not effective for the conversion of **6**.

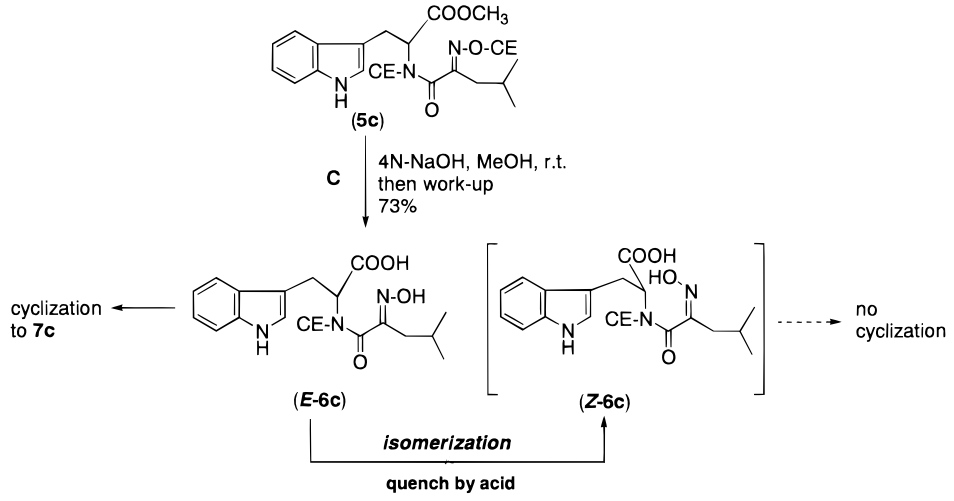
(5) See for example: *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley & Sons: New York, 1995; pp 3609−3611.

(6) The cyclized product **7a** was susceptible to hydrolysis back to the carboxylic acid **6a**. ^{1}H NMR spectra of the recovered **6** indicated the presence of (*E*)- and (*Z*)-isomers of the oxime.

Scheme 4. **Improved route for plant-scale synthesis**



Scheme 5. ***E,Z*-Isomerization of the oxime**



methylation reaction, DMF was a preferable solvent to dioxane. Thus, the protection of the N^1-position opened a simple and selective protocol for cyclization and *O*-methylation without any isomeric product. This was previously

unattainable without such protection (compare with Scheme 3).

We then had to investigate the deprotection process. Careful attempts to deprotect **8a** with TFA in the presence of anisole caused an undesirable migration of the 2,4-DMPM group to the 2-position of the indole ring at a considerable ratio to produce the side product **9**. Various scavengers and acidic conditions were then surveyed but none of them could suppress the migration of 2,4-DMPM group.[7] Our best result, in which **1** was obtained in 50% (with 18% reduced yield for **9**), was realized by the combination of TFA and *m*-cresol.

Unfortunately, it was impossible to isolate **1** in a sufficiently pure form without using chromatography, which led us to use other protecting groups as described below (Scheme 4).

Protection by 2-Phenylsulfonylethyl (PSE) Group. We then experimented with base-labile (base-removable)-protecting groups such as 2-phenylsulfonylethyl (**PSE**) and 2-cyanoethyl (**CE**) groups. On treatment of **5b** (P═PSE, prepared from **3b** and PSE-Br) with NaOH in MeOH, both the methyl ester and the CE group of oxime were found to be hydrolyzed selectively, while the N^1-protecting group remained intact (nearly quantitative yield). Then, cyclization of **6b** with DCC−HOSu in DMF and successive methylation with K_2CO_3- $(CH_3)_2SO_4$ gave the expected product **8b** in 40% yield. Further treatment of **8b** with 1 N NaOH in MeOH gave **1** in 68% yield. Fortunately enough, this one-pot process (cyclization−methylation−deprotection) was possible by treating the unnecessary reagent $(CH_3)_2SO_4$ with MeOH before the removal of the N^1-protecting group. The whole operation proceeded more easily than before. The yield of the last three steps in one-pot was 36% with DCC−HOSu cyclization, while a slightly decreased yield of 34% was attained with DCC only.

These synthetic operations with PSE group showed that transformation under mild basic conditions were suitable for the pyrazine ring manipulation. However, its introduction was not regarded as economic.

Protection by the 2-Cyanoethyl (CE) Group: A Final Tuning. We next moved to investigate some advantages of a CE group over the expensive PSE group. The protection and deprotection of CE group could be attained under conventional basic conditions as detailed in the Experimental Sections. This was by far more effective and simple than the above two protecting groups.

Thus, **5c** was prepared from **3c**, which was then selectively converted to the cyclization precursor **6c** without difficulty under the alkaline hydrolysis conditions. The oxime-CE group was removed, while the N^1-CE group remained intact. After the one-pot transformation (cyclization, methylation, and deprotection) under alkaline conditions, **1** was isolated in 49% yield from carboxylic acid **6c** with DCC−HOSu as a condensing agent. In this DCC-cyclization step, effects of other additives were investigated. Following yields were obtained after one-pot transformation to **1** with different additives to DCC; i.e., *p*-NO_2PhOH (51%), PhOH (51%), (*iso*-Pr)$_2$NH (40%) and 58% without such additives.

Having arrived at the simple and effective plant route, final tuning was required at the last stage to attain high reproducibility with simplest operation.

At the oxime deprotection step, careful pH adjustment in the workup to prevent acid-catalyzed isomerization at the oxime nitrogen was essential. The mixture was treated under controlled pH to make rapid precipitation of the free oxime (***E*-6c**) from the mixture, thus preventing the undesired isomerization at the oxime nitrogen (which sometimes occurred in the acidic media). Only one oxime isomer (*E*-oxime, i.e., ***E*-6c**) is suitable for pyrazine cyclization, and the corresponding *Z*-isomer (***Z*-6c**) is not, because of its steric nature.

Thus, starting from tryptophan methyl ester (**3**) and ketoleucine oxime, the highest overall yield of 40% was attained for **1**, with adequate care in the workup stage of oxime deprotection and cyclization. HPLC purity of crude **1** (after triturating with AcOEt) obtained in this way was more than 99.5%. The crude product thus obtained was further purified by the following two-step purification: (i) formation of the DMF complex (90.3%), (ii) recrystallization of the DMF complex from EtOH (91.2%). The final product was obtained with 99.9% purity as judged by HPLC analysis.

Summary

The process research described herein highlights the beneficial use of a protecting group in plant synthesis. A careful selection of the suitable nitrogen protecting group as well as additional tunings for simple and effective transformation and purification led us to the most reliable process to **1**. Our present synthesis includes the following advantages over the previous routes: (1) satisfactory overall yield, (2) the shortest number of steps, (3) convenient reagents employed in methylation, (4) no chromatography required throughout the process, (5) the simple procedures for protection−deprotection sequence suitable for large scale production.

One-pot reactions that involve multiple steps are of general importance in organic synthesis, which is one of our current concerns. We are working on the possibility of the shortest entry into the pyrazine skeletons. In addition to these chemistries associated with plant synthesis, some other successful applications to the natural pyrazines have been attained and are briefly summarized in our recent review (in Japanese),[8] full detail of which will be reported elsewhere in due course.

Experimental Section:

General Procedures. Reagents and solvents were used as received (reagent grade) without further purification. Reactions were usually conducted under nitrogen as indicated. All of the melting points were uncorrected. TLC analysis was carried out using Merk silica gel 60 F_{254} plate

(7) The migration of the 2,4-DMPM group to the 2-position of the adjacent indole ring under acidic conditions was found to be avoided by protection of indole-NH with Boc or acetyl group.

(8) Matoba, K.; Tone, H.; Shinhama, K.; Goto, F.; Saka, M.; Minamikawa, J. *J. Synth. Org. Chem. Jpn.* **1999**, *57*, 407−414.

(Art 5715). Analytical determinations by HPLC were performed on a Shimadzu LC-6A liquid chromatography with a TSK gel ODS-80TM column. ^{1}H NMR spectra were taken at Varian 200 MHz or 300 MHz spectrometer, and selected distinctive peaks are recorded, owing to the complexity of the rotational isomers. IR spectra were recorded with a Perkin-Elmer 1600 series FTIR apparatus. Mass spectra were recorded with a Shimadzu GCMS-QP1000 spectrometer at 70 eV.

***N*-Protected L-Tryptophan Methyl Ester Hydrochloride (3c).** Neat Et_3N (7.1 L) was slowly added to a stirred solution of L-TrpOMe-HCl (13.0 kg) in MeOH (78.0 L) under N_2 to form a clear solution (at pH = 7−8). Acrylonitrile (16.8 L) was then slowly added below 20 °C. After initial exothermic process, the mixture was refluxed for 7−8 h. The resulting mixture was kept standing at room temperature. for ∼15 h (TLC analysis indicated the complete consumption of the starting material). The mixture was carefully concentrated under reduced pressure (below 35 °C), during which time precipitate formed gradually. After azeotropic drying by addition of 2-propanol (IPA, 130 L), the residue was suspended in IPA (130 L) and acidified with concentrated HCl (5.15 L) below 20 °C. The precipitate formed was kept below 10 °C for 1 h. The resulting matured crystal was then filtered and washed with IPA (39.0 L). The thus obtained wet product (21.5 kg) was recrystallized from IPA (39.0 L) −H_2O (19.5 L) at 80−10 °C (finally 10 °C, 1 h). The wet material (17.5 kg) filtered was further dried at 60 °C for 15 h, furnishing the title compound **3c** (13.82 kg; 87.9%). **3c:** mp 178−80 °C; IR (neat) 3404, 1730, 1433, 745 cm^{-1}; ^{1}H NMR

($CDCl_3$−CD_3OD) δ 8.33 (1 H, brs), 7.58 (1 H, d, J = 10 Hz), 6.97−7.33 (4 H, m), 3.65 (3 H, s), 3.60 (1 H, m), 3.19, 3.08 (each 1 H, m), 2.91, 2.68 (each 1 H, d, J = 6 Hz), 2.32 (2 H, t, J = 6 Hz); MS (271, M^+). Anal. Calcd for $C_{15}H_{18}ClN_3O_2$: C, 58.53; H, 5.90; N, 13.65. Found: C, 58.55; H, 5.87: N, 13.75.

***N*-Protected Leucine Oxime (4p).** To a cooled (∼10 °C) and stirred solution of ketoleucine oxime (10.0 kg) in DMF (50.0 L) was added aqueous NaOH solution (5.51 kg NaOH in 25.0 L H_2O), while keeping the temperature below 25 °C. To the resulting clear solution was added acrylonitrile (22.8 L) under adequate cooling (15 °C). After the exothermic process (30−35 °C), the mixture was stirred at 30−35 °C for 7.5 h and kept standing at room temperature. (TLC analysis confirmed the complete reaction with $CHCl_3$: MeOH: Et_3N = 5:1:1). The crude mixture was then cooled to 10 °C before adding 3 N HCl (70 L) below 20 °C to adjust pH = 3−4. Dilution and extraction with AcOEt (80 L)−10% NaCl (20 L) followed by re-extraction (AcOEt: 50 L × 2) afforded the crude extracts, which was subsequently washed with 10% NaCl aq (50 L × 3, pH = 3) to give a clear organic layer (HPLC analysis). Evaporation of the extracts after drying over $MgSO_4$ (1.5 kg) gave an oily material **4p** (11.34 kg, 83.0%), which was kept under N_2 for the next condensation. **4p:** mp 52 °C; ^{1}H NMR ($CDCl_3$) δ 4.78 (1 H, brs), 4.42, 2.82 (each 2 H, t, J = 7 Hz), 2.50 (2 H, d, J = 7.5 Hz), 2.15−1.95 (1 H, m), 0.94, 0.91 (each 3 H, d, J = 6.5 Hz). Anal. Calcd for $C_9H_{14}N_2O_3$: C, 54.53; H, 7.12; N, 14.13. Found: C, 54.48; H, 7.06; N, 14.07.

Protected Amide (5c). *(1) Preparation of the Segments.* The *N*-protected L-tryptophan methyl ester hydrochloride **3c** (13.28 kg) was added to a stirred solution of K_2CO_3 (41.74 kg) in H_2O (93 L)-CH_2Cl_2 (93 L) below 25 °C (7−17 °C) with stirring to form a clear layer of the free *N*-protected tryptophan ester (**A**), which was maintained under 10 °C (4−9 °C) until the next condensation reaction.

On the other hand, neat $POCl_3$ (5.31 L) was added carefully to a cooled solution (0−4 °C) of the protected oxime **4p** (10.26 kg) in CH_2Cl_2 (53 L)−DMF (4.4 L), while the temperature was raised to 30−35 °C. After stirring for1−2 h (HPLC analysis), the resulting solution (**B**) of the acid chloride was cooled to 4−9 °C for the next condensation with the free base **3c**.

(2) Amidation. The solution (**B**) was slowly added to the above prepared solution (**A**) below 20 °C (4−19 °C). The mixture was stirred for 0.5 h, and TLC analysis indicated the complete consumption of the starting material. The mixture was quenched by the addition of H_2O (80 L) and the organic layer was separated. The aqueous layer was re-extracted with CH_2Cl_2 (66 L), and the combined organic layer was treated with aqueous K_2CO_3 (K_2CO_3 0.6 kg in 66 L H_2O), followed by washing with dilute HCl (concentrated HCl 16.6 L in 50 L H_2O) and H_2O (66 L). The separated organic layer was then concentrated to afford a crude oil, which was crystallized from IPA (64 L)-H_2O (43 L) at 70−35 °C. After cooling under 5 °C for 1 h, crystalline solid was filtered and washed with IPA (40 L) to leave the protected amide (**5c**, 17.82 kg, wet) which was dried at 60 °C for 15 h, furnishing the pure material (17.18 kg, 88.2%). **5c:** mp 101−103 °C; IR (KBr): 3397, 2960, 1726, 1634, 1434, 744 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.18 (s, 0.7 H), 8.13 (s, 0.3 H), 7.55 (d, 1 H, J = 7.5 Hz), 7.39 (d, 1 H, J = 7.5 Hz), 7.23 (t, 1 H, J = 7.5 Hz), 7.13 (t, 1 H, J = 7.5 Hz), 7.02 (d, 1 H, J = 2.5 Hz), 5.20 (dd, 0.3 H, J = 5.5, 9 Hz), 4.30 (t, 1.4 H, J = 6 Hz), 4.12, 4.03 (dt, each 0.7 H, J = 2, 6.5 Hz), 3.79 (s, 3 H), 3.63−3.92 (m, 0.7 H), 3.60 (d, 1.4 H, J-8.5 Hz), 3.36−3.57 (m, 1.4 H), 3.20 (dd, 0.3 H, J = 9, 16 Hz), 2.86 (t, 0.7 H, J = 7.5 Hz), 2.65 (t, 1.4 H, J = 6 Hz), 2.42−2.64 (m, 2 H), 2.03−2.26 (m, 1 H), 1.78−2.03 (m, 1.7 H), 1.57−1.75 (m, 0.3 H), 0.93 (d, 4.2 H, J = 6.5 Hz), 0.70, 0.72 (d, each 0.9 H, J = 6.5 Hz); MS (451, M^+). Anal. Calcd for $C_{24}H_{29}N_5O_4$: C, 63.84; H, 6.47; N, 15.21. Found: C, 63.82; H, 6.41; N, 15.41.

Oxime Acid (6c). An aqueous NaOH solution (0.31 kg NaOH in 1.75 L H_2O) was added to a stirred solution of **5c** (0.7 kg) in MeOH (3.5 L) under cooling (∼10 °C). The resulting mixture was kept stirring at 25−30 °C until TLC indicated complete consumption of SM (∼2 h, clear solution). The mixture was cooled to 15 °C and diluted with H_2O (7 L) for workup. Thus, the cooled (15 °C) solution of **6c** obtained was carefully neutralized by the dropwise addition to a cold HCl solution (0.82 L concentrated HCl−3.5 L H_2O−1.75 L MeOH) containing a small amount of a seed crystal of **6c**. An exothermic reaction was observed while the mixture was kept at 5−15 °C for 0.5h to give a

precipitate, which was filtered and washed with H_2O (7 L × 2). The wet material (0.83 kg) obtained after filtration was dried at 60 °C until H_2O content became constant (2.0−2.6 ppm after 17−20 h) to furnish the cyclization precursor **6c** (570 g, 95.64%). **6c:** mp. 117−20 °C; IR (neat) 3404, 1730, 1433, 745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.91(2 H, brs), 8.47 (1 H, brs), 7.7−6.9 (5 H, m), 3.62, 2.62 (each 2 H, t, J = 10 Hz), 1.0−0.6 (6 H, m); ^1H NMR (DMSO-d_6) δ 12.60 (brs, 1 H), 11.48 (s, 0.7 H), 11.44 (s, 0.3 H), 10.93 (s, 0.3 H), 10.88 (s, 0.7 H), 7.49 (d, 0.3 H, J = 8 Hz), 7.34 (d, 1 H, J = 8 Hz), 7.10 (t, 1 H, J = 8 Hz), 7.05 (d, 1 H, J = 2.5 Hz), 6.99 (t, 1 H, J = 8 Hz), 5.04 (dd, 0.3 H, J = 5.5, 7.5 Hz), 4.46 (dd, 0.7 H, J = 5.5, 7.5 Hz), 3.68 (t, 0.6 H, J = 7.5 Hz), 3.14−3.61 (m, 2.7 H), 2.93−3.14 (m, 0.7 H), 2.78 (t, 0.6 H, J = 7.5 Hz), 2.13−2.57 (m, 1.4 H), 2.34 (d, 1.4 H, J = 7 Hz) 1.70−2.07 (m, 1 H), 1.37−1.60 (m, 0.6 H), 0.79, 0.82 (d, each 2.1 H, J = 6 Hz), 0.54, 0.60 (d, each 0.9 H, J = 6 Hz); MS (366, $[M - H_2O]^+$). Anal. Calcd for $C_{20}H_{24}N_4O_4 \cdot {}^1/_2H_2O$: C, 61.05; H, 6.40; N, 14.24. Found: C, 61.28; H, 6.25; N, 14.15.

One-pot Synthesis of OPC-15161(1) from 6c. The oxime acid **6c** (3.72 kg) was added to a stirred solution of DCC (2.2 kg) in DMF (26.0 L) at room temperature. and the resulting mixture was kept stirring at 20−25 °C for 1 h. TLC analysis indicated complete consumption of **6c** with exclusive formation of **7c**. This crude mixture of **7c** was cooled to 15−20 °C, before the addition of Me_2SO_4 (2.75 L) and Na_2CO_3 (3.08 kg) in this order. Exothermic reaction took place with gas evolution. The mixture was kept stirring for at 25−30 °C for 3−5 h. TLC analysis then indicated the exclusive formation of the methyl ether (**8c**) with complete conversion from **7c**. The crude product **8c** was maintained at 15−20 °C, before quenching the excess Me_2SO_4 by addition of MeOH (26 L) below 25 °C. The resulting mixture was then treated with aq. NaOH solution (1.95 kg NaOH in 12 L H_2O) below 25 °C. The resulting mixture was kept stirring at 20−25 °C for 2 h. After standing overnight, the insoluble precipitate was first filtered off and washed well with H_2O (14.9 L). The combined filtrate was cooled below 15 °C and carefully acidified by concentrated HCl (6.45 L) under stirring. After stirring for 1 h, the crude precipitate (**A**) was filtered and washed with H_2O (11.5 L). All of the filtrate was extracted with AcOEt (56 L × 2), and the combined organic layers were washed with H_2O (37 L × 2). The organic layer was dried over Na_2SO_4 and concentrated to less than 80 L under reduced pressure. To this residue was added the crude precipitate (**A**) and AcOEt (37.2 L). The whole mixture was kept stirring at 15−20 °C for 1−1.5 h to form crude precipitate of **1**. The ppt was filtered and washed with AcOEt (11.5 L) to leave wet material (2.26 kg), which on drying at 60 °C for 15 h afforded the nearly pure compound **1** (2.18 kg; 68.8% from **6c**) with 99.5% purity judged by HPLC.

OPC-15161 DMF Complex. The product obtained as above (**1**, 4.26 kg) was dissolved in DMF (14.90 L) and AcOEt (6.40 L) with stirring. The mixture was gradually heated to ∼80 °C to form a clear solution. The resulting solution was gradually cooled to −5 °C to obtain the precipitate, which was collected by filtration and dried at 60 °C for more than 15 h to leave the DMF complex (4.71 kg, 90.38%) with 99.9% purity judged by HPLC.

Recrystallization from EtOH. The DMF complex prepared as above (9.09 kg) was dissolved in EtOH (218 L) and H_2O (55 L) with stirring. The mixture was gradually heated to ∼80 °C to form a clear solution. This solution was filtered while it was hot and the filtrate was heated again at ∼80 °C to form clear yellow solution. The solution was then subjected to crystallization under careful temperature control (55 °C, 1 h; 50−40 °C, 1 h; 40−30 °C, 1 h; 30−20 °C, 1 h; 20−10 °C, 1 h and finally 10−0 °C, 1 h). The precipitate was filtered and dried at 60 °C for 15 h to afford the title compound (**1**, 6.78 kg; 91.2%) with 99.9% HPLC purity. **1:** mp. 225 °C; IR (KBr) 3438, 1716, 1636, 1456, 1379, 1255, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.96 (1 H, br s), 10.94 (1 H, s), 7.57 (1 H, d, J = 7.9 Hz), 7.34 (1 H, d, J = 7.9 Hz), 7.24 (1 H, brs), 7.07 (1 H, t, J = 7.9 Hz), 6.99 (1 H, t, J = 7.9 Hz), 3.92 (2 H, s), 3.77 (3 H, s), 2.60 (2 H, d, J = 7.2 Hz), 2.09 (1 H, qt, J = 7.2, 6.6 Hz), 0.85 (6 H, d, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6) δ 156.6, 143.4, 138.7, 136.4, 132.1, 126.9, 123.9, 121.2, 118.7, 111.6, 109.9, 60.7, 33.0, 25.5, 24.5, 22.7; MS (327, M^+). Anal. Calcd for $C_{18}H_{21}N_3O_3$: C, 66.03; H, 6.47; N, 12.84. Found: C, 66.00; H, 6.50; N, 12.79.

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